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Evolution of Fast Development of Planktonic Embryos to Early Swimming

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Abstract. Planktonic embryos of marine animals swim at an early stage and age. Although natural selection has apparently favored rapid development of structures for swimming, taxa have not converged on the same, minimal time from first cell division to first swimming. Comparisons of 34 species with planktonic embryos in 10 phyla revealed factors that account for variation in time to swimming. Time to first swimming correlated significantly with time from first to second cleavage (first cell cycle) in analyses of all embryos sampled and separately within the Spiralia and Echinodermata. Time to first swimming also correlated significantly with egg diameter in some clades, but not in all. Correlations between egg diameter and cell cycle duration were low except for the three species of Urochordata. Development to a feeding or nonfeeding larva did not affect time to first swimming beyond effects attributable to egg size. Time to first swimming did not correlate with type of locomotion developed (uniciliated cells, multiciliated cells, or muscle). Nonetheless, differences in locomotion are associated with changes in cell cycle durations prior to swimming. The ratios of time to first swimming and time for first cell cycle suggests that allocation of time to multiplication of cells *versus* differentiation of cells is resolved differently in species with different types of locomotion.

Introduction

Many marine invertebrates release their eggs individually into the plankton, where each embryo develops with little protection. A common feature of such embryos is rapid development of locomotion. We expect that the factors influencing age and stage at first swimming have influenced the evolution of most animal embryos. Multicellular animals originated and diverged into most major existing clades in the sea, and development *via* small, solitary embryos is inferred to be an ancient and persisting mode of development. We also expect that planktonic development of more recent origin has converged to some extent on similar early swimming. Here we examine the degree of convergence and divergence in rates of development and time to first swimming by comparing diverse planktonic embryos.

Solitary embryos are at risk. Embryos contain rich nutrient stores for predators but are more limited than larvae in means of defense or flight. Although planktonic embryos have extra-embryonic envelopes or coats (Strathmann, 1987), some contain toxins (Lindquist, 1996; McClintock and Baker, 1997), and the plankton may be a safer environment than the bottom, planktonic embryos lack the parental care, protective gel, or tough envelope that shields most benthic embryos. Planktonic embryos are therefore among the least protected and most vulnerable embryos of marine animals.

Sources of mortality for planktonic embryos include predation (Pennington and Chia, 1984); ultraviolet radiation (Morgan and Christy, 1996; Epel *et al.*, 1999); advection from suitable adult habitat (Jackson and Strathmann, 1981); and deposition on the bottom, where risks may be even greater for single embryos. Although mortality rates for planktonic embryos have not been estimated, they are presumably at least as great as those of small larvae (Pennington *et al.*, 1986). Estimates of instantaneous mortality rates for small planktonic larvae are high, ranging from 0.04 to 1.0 per day (Strathmann, 1985; Rumrill, 1990).

There are no obvious benefits from prolonging planktonic embryonic development. Planktonic embryos have limited opportunities for growth or reproduction. Active transport

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of dissolved organic molecules from the environment is well documented in larvae (Manahan and Crisp, 1982; Jaeckle and Manahan, 1989; Manahan, 1990) and may be common in embryos (Chia, 1972; DeBurgh and Burke, 1983; Manahan, 1983; Shilling and Bosch, 1994). However, there is little growth before a functional gut develops and feeding begins (Strathmann, 1987; Fenaux *et al.*, 1994).

With no apparent benefit and substantial risk to prolonging planktonic embryonic development, natural selection should favor a reduction in the duration of this high-risk life-history stage (Shine, 1989). Comparisons of early embryonic cell cycle durations of planktonic and protected benthic embryos in several independent evolutionary divergences confirm that cell cycles are shorter in planktonic embryos and that the range of cell cycle durations is less for the planktonic than for the benthic protected embryos (Strathmann *et al.*, 2002). The vulnerability of planktonic embryos is indeed associated with rapid development.

Comparisons also indicate that selection has favored early swimming in development of planktonic embryos. Developmental times prior to first swimming in planktonic embryos are especially short. Planktonic embryos swim at earlier stages than more protected embryos, and the structures in the first locomotory stages are less developed in broadcasting species than in species that brood or deposit egg masses (Strathmann, 1987; Kiørboe and Sabatini, 1994; Satoh, 1994; Strathmann *et al.*, 2002). Development of locomotory cilia is one of the earliest morphogenetic activities of the zygotic genome in diverse planktonic embryos. In spiralian embryos, the trochoblasts are the first cells to differentiate during embryonic development (Kooij *et al.*, 1998). Ciliogenesis is the first zygotically programmed morphogenetic event in sea urchin embryos (Stephens, 1995). First swimming occurs at different stages of development in different planktonic embryos (Strathmann, 1987). Of ciliary swimmers, some asteroids first swim as blastulae, others as gastrulae; spiralians swim before or after gastrulation, or even as veliger larvae. Some must develop muscles, as in the tunicate tadpole. However, in all these groups, planktonic development of single embryos is associated with earlier development of locomotory structures relative to other structures.

Although comparative evidence for advantages to early swimming in planktonic development is strong, the costs and benefits of early swimming, as opposed to passively sinking or floating, are unclear. Swimming, if faster than sinking, could increase encounters with ambush predators (Gerritsen and Strickler, 1977; Gerritsen, 1980), but swimming may also confer some protection against capture by some pelagic predators (Pennington *et al.*, 1986). Moreover, models of vertical swimming by flagellates in a wind-mixed water column (Yamazaki and Kamykowski, 1991) indicate that even the slow upward swimming of sea urchin blastulae (Mogami *et al.*, 1988) should often reduce encounters with benthic predators, relative to the slow downward sinking prior to swimming. The quantitative effects of swimming depend on swimming speed, adjustments in orientation, and rates of turbulent mixing, but first swimming is nevertheless an important life-history event. Prior to swimming, these embryos drift passively. Most sink slowly in still water, while some larger embryos float upwards. When they begin to swim, they can adjust their position in the water column (Yamazaki and Kamykowski, 1991; Eckman *et al.*, 1994; Kelman and Emlet, 1999; Metaxas, 2001). First swimming marks a transition into a new set of capabilities and selective pressures.

To examine trade-offs and constraints that limit the evolution of rapid embryonic development, we examined time to swimming of disparate, distantly related planktonic embryos. We took similarity in time as a measure of convergence. We investigated egg size, cell cycle duration, type of locomotion, and larval nutrition as factors that could account for the variation. Correlations of these factors with time to first swimming then point to limits on convergence of distantly related embryos.

Egg size

Some studies have reported longer times to swimming in species with larger eggs (*e.g.*, tunicates: Berrill, 1935; copepods: McLaren, 1966; fish: Duarte and Alcaraz, 1989; gastropods: Kohn and Perron, 1994). Others have found no relationship (echinoids: Dickie *et al.*, 1989; asteroids: Hoegh-Guldberg and Pearse, 1995; copepods: Kiørboe and Sabatini, 1995). Berrill (1935) and McLaren (1966) interpreted exceptions to the trend of longer times with larger eggs as the result of differences in "yolkiness," as indicated by optical density. Berrill (1935) also found a significant correlation between egg size and cell cycle duration in tunicates, which could partially explain the correlation he found between egg diameter and time to swimming. Among species that swim with cilia, time to first swimming might correlate with egg diameter if larger embryos need more cilia for propulsion (Emlet, 1994) and if there is a time constraint to building many cilia.

Cell cycle duration

The duration of the first cell cycle (first to second cleavage) may correlate with time to first swimming if cell multiplication is a primary factor limiting rapid development to swimming. The first cell cycle is among the fastest in an animal's life history. The durations of other early cell cycles are nearly proportional to the durations of this first cell cycle; thus the first cell cycle is representative of others in early development, within a species at different temperatures (Dettlaff, 1964) or between closely related species (Schneider *et al.*, 1992). Although there is some early transcription of the zygotic genome in at least some planktonic

embryos (Davidson, 1986), the earliest cell cycles of diverse rapidly developing embryos include synthesis of new DNA and mitosis without measurable gap phases (Murray and Hunt, 1993). Differences in early cell cycle duration may result from constraints or trade-offs at a molecular level (Strathmann *et al.*, 2002).

Type of locomotion

Planktonic embryos swim in disparate ways, using very different structures (Chia *et al.*, 1984). The locomotory structures at first swimming can be very conservative traits, unchanged in many lineages since the divergence of phyla and classes. Echinoderms swim as uniformly ciliated blastulae or gastrulae with only one cilium on each cell. Most spiralians (annelids, molluscs, nemerteans, etc.) form specialized regions of cilia for propulsion, with many cilia on each cell. Other animals, such as tunicates and crustaceans, start to swim using muscle. The type of locomotory structure that an embryo differentiates might affect the time for development to a swimming stage. For example, embryos with many cilia per cell might be expected to swim with fewer cells and perhaps earlier than embryos that have only one cilium per cell. Conversely, embryos that incorporate multiciliated cells into specialized swimming structures (*e.g.*, ciliated bands or ctene rows) might be expected to take longer to construct their swimming apparatus than embryos that swim with simple, uniciliated cells. Animals that swim with muscles might be expected to swim later than those that use cilia, because they must develop more complex tissues.

Mode of larval nutrition

Some embryos develop into larvae that swim and feed in the plankton. Others form nonfeeding larvae and rely primarily on nutrients stored in the egg for energy (Thorson, 1950). Embryos that develop into feeding larvae might be expected to take longer to develop to swimming if developing feeding structures were to compromise development of early locomotion. Conversely, the benefit of earlier feeding might outweigh other costs of rapid embryonic development and result in earlier swimming. Or there could be no effect of larval feeding because species with feeding larvae and planktonic development start to swim well before they start to feed (Strathmann, 1987).

Materials and Methods

We investigated the embryonic development of 34 species in 10 phyla (Table 1). Species identification followed Kozloff (1987). Identification of *Henricia leviuscula* and *Henricia* (gray armpit) followed M. Strathmann (pers. comm., 1998). *Henricia* (gray armpit) is an undescribed species with a distinctive color pattern. All animals were collected in the San Juan Archipelago and nearby areas of Washington State and are native to the region. Methods for obtaining eggs and sperm and rearing embryos are described in Strathmann (1987), except that zygotes of *Membranipora membranacea* were obtained by dissection, as in the method for *Phoronis vancouverensis*, and spawning of the protobranch bivalve *Acila castrensis* followed Zardus and Morse (1998).

Inseminated eggs were distributed into ~80 ml of 0.45-μm filtered seawater. Developing embryos in beakers were incubated in water baths at 10, 14, and 18 °C (accurate to within ± 0.3 °C). Since many embryos did not develop to swimming at 18 °C, data only from 10 °C and 14 °C will be discussed here. Seasonal and geographic comparisons indicate that although temperature tolerances vary within species, there is little acclimation (Fujisawa, 1995; Nomaguchi *et al.*, 1997) or adaptation (Bosch *et al.*, 1987; Hoegh-Guldberg and Pearse, 1995) of development rates. Selection at two temperatures produced little change in duration of pupal stages of fruit flies (Partridge *et al.*, 1994). Nevertheless, temperature acclimations have been reported (copepods: Landry, 1975; Hart and McLaren, 1978; Tester, 1985; echinoids: Johnson *et al.*, 1990). We therefore compared rates for embryos from one region and at two temperatures to avoid unrecognized bias from acclimation or adaptation.

In most cases a sufficient number of embryos was obtained from a single pair of parents (or one self-fertile hermaphroditic parent) to divide between the two temperatures. We limited the number of embryos per beaker so that development rates would not be oxygen limited (Strathmann and Strathmann, 1995). Video cameras mounted on dissecting microscopes over the water baths and a time-lapse recorder recorded development of the embryos. Accuracy in time measurements was limited to 1.5 min.

For each group of embryos, we measured the diameter of about 10 eggs and recorded the mean. For some cases there was more than one spawn, and the spawn means were averaged as an estimate of egg size for the species. In some cases our reported mean egg diameter for a species differs slightly between temperatures because different groups of embryos were observed at each temperature.

Hatching and swimming do not coincide in all species. Polychaetes often incorporate the egg envelope into the larva and thus never truly hatch (Strathmann, 1987). The appendicularean tadpoles hatched and then twitched as long as 45 min before they swam. In all but one species (*Calliostoma ligatum*), we defined first swimming as the time when an embryo moved away from its position, though many rotated in place before swimming. We recorded the time to rotation instead of time to swimming for *C. ligatum*. Trochophores of the gastropod *C. ligatum* rotate in capsules several days at 12 °C before hatching as veligers (Strathmann, 1987). For each treatment of embryos, we recorded the time from first to second cleavage and the time from first

Table 1

Age and stage at first swimming and other features of development for 34 species with planktonic embryos

PHYLUM Class *Species*	Mean egg diameter (μm)		Time from 2-cell to 4-cell (h)		Time to first swimming (h)[1]		Type of locomotion[2]	Mode of larval nutrition[3]	Stage at first swimming[4]
	10 °C	14 °C	10 °C (*n*)	14 °C (*n*)	10 °C (*n*)	14 °C (*n*)			
CNIDARIA									
Aglantha digitale	142.0	136.4	1.38 (18)	0.97 (17)	10.53 (19)	7.72 (17)	UC	NF	planula
Mitrocoma cellularia	153.1	153.1	1.28 (15)	0.85 (15)	16.73 (15)	10.77 (15)	UC	NF	planula
CTENOPHORA									
Pleurobrachia bachei	144.0		0.95 (6)		28.47 (4)		MC	F	cydippid
NEMERTEA									
Micrura alaskensis	69.7	69.7	1.52 (18)	0.92 (25)	29.95 (16)	16.43 (25)	MC	F	blastula* (a)
ANNELIDA									
Polychaeta									
Owenia fusiformis	68.6	71.3	0.88 (8)	0.63 (17)	29.33 (11)	18.23 (23)	UC	F	trochophore
Serpula columbiana	68.0	67.9	1.20 (13)	0.66 (14)	12.90 (10)	7.35 (19)	MC	F	trochophore
Arctanae fragilis	75.8	75.8	1.00 (13)	0.65 (10)	9.27 (13)	6.42 (8)	MC	F	trochophore
Sabellaria cementarium	77.5	77.5	1.58 (9)	0.92 (5)	17.28 (8)	10.72 (5)	MC	F	gastrula*
MOLLUSCA									
Bivalvia									
Mytilus trossulus	61.2	61.2	1.22 (13)	0.75 (8)	11.28 (13)	7.02 (10)	MC	F	blastula*
Chlamys hastata	68.1	67.7	1.71 (23)	1.04 (23)	27.96 (22)	17.53 (23)	MC	F	gastrula*
Acila castrensis	126.0	126.0	2.18 (5)	1.47 (4)	40.65 (5)	25.2 (4)	MC	NF	blastula* (b)
Polyplacophora									
Mopalia muscosa	204.7	206.0	1.37 (9)	0.80 (7)	36.30 (18)	21.12 (8)	MC	NF	gastrula*
Gastropoda									
Tectura scutum	138.2	138.2	0.87 (10)	0.55 (9)	19.77 (10)	14.72 (10)	MC	NF	trochophore
Calliostoma ligatum	233.1	233.1	1.57 (10)	1.03 (6)	41.32 (9)†	26.13 (5)†	MC	NF	veliger
PHORONIDA									
Phoronis pallida	62.6	62.6	1.25 (6)	0.68 (7)	11.03 (6)	6.37 (7)	UC	F	blastula (c)
BRACHIOPODA									
Terebratalia transversa	160.2	160.2	1.03 (10)	0.63 (14)	14.25 (8)	10.65 (13)	UC	NF	blastula
BRYOZOA									
Membranipora membranacea	60.2	59.6	1.38 (16)	0.87 (7)	32.96 (14)	18.43 (3)	MC	F	early larva (c)
ECHINODERMATA									
Echinoidea									
Strongylocentrotus purpuratus	85.8	85.8	1.95 (8)	1.40 (7)	23.63 (11)	17.12 (9)	UC	F	blastula
Strongylocentrotus franciscanus	131.2	131.2	1.58 (12)	1.05 (12)	22.88 (14)	14.97 (13)	UC	F	blastula
Strongylocentrotus droebachiensis	163.4	163.4	1.48 (22)	1.09 (12)	22.25 (29)	17.22 (13)	UC	F	blastula
Dendraster excentricus	130.0	130.0	1.67 (15)	0.97 (12)	23.22 (15)	13.63 (13)	UC	F	blastula
Holothuroidea									
Parastichopus californicus	191.3	191.3	2.03 (12)	1.27 (5)	40.72 (12)	25.52 (5)	UC	F	blastula
Asteroidea									
Luidia foliolata	152.0	152.0	1.93 (7)	1.13 (11)	39.92 (10)	25.63 (11)	UC	F	early gastrula (d)
Orthasterias koehleri	148.2	148.2	2.00 (7)	1.40 (8)	39.73 (7)	27.63 (10)	UC	F	blastula
Evasterias troschelii	162.5	162.5	1.50 (3)	1.18 (9)	32.23 (5)	21.95 (6)	UC	F	blastula
Pisaster ochraceus	178.0	177.5	1.57 (8)	1.13 (6)	33.95 (8)	22.43 (6)	UC	F	blastula
Crossaster papposus	796.5		2.03 (5)		57.12 (5)		UC	NF	gastrula (d)
Pteraster tesselatus	1175.0		1.68 (6)		63.32 (3)		UC	NF	gastrula (e)
Henricia (grey armpit)	1087.0		1.73 (6)		65.12 (6)		UC	NF	gastrula (d)
Henricia leviuscula	1342.0		1.98 (8)		72.53 (8)		UC	NF	gastrula (d)
Ophiuroidea									
Ophiophalis aculeata	96.5	96.5	1.45 (7)	1.20 (4)	18.93 (7)	14.22 (5)	UC	F	blastula
UROCHORDATA									
Appendicularia									
Oikopleura dioica	76.0	76.0	0.33 (4)	0.23 (9)	8.87 (7)	6.00 (4)	M	NF	tadpole
Ascidiacea									
Boltenia villosa	155.7	156.9	0.92 (12)	0.59 (10)	30.62 (13)	18.75 (16)	M	NF	tadpole
Ascidia paratropa	169.3	167.9	1.08 (13)	0.70 (12)	45.80 (17)	28.92 (10)	M	NF	tadpole

[1] If more than one batch of eggs was observed per species, the mean of the median times to swimming is reported.

[2] UC = uniciliated cells; MC = multiciliated cells; M = muscle.

[3] NF = nonfeeding larvae; F = feeding larvae.

[4] Stages at swimming are from Strathmann (1987) except (a) S. Stricker (Univ. of Washington, pers. comm); (b) Zardus and Morse (1998); (c) R. Zimmer (Univ. of Southern California, pers. comm); (d) unpubl. obs.; (e) Kelman and Emlet (1999).

* Most of the spiralians began to swim as trochophores (with ciliated trochoblasts). Some sources indicate stage with regard to gastrulation.

† Times to rotation are reported for *Calliostoma ligatum*.

cleavage to first swimming for a minimum of 3 and a maximum of 15 embryos. Cleavage furrows were at similar stages for recorded times for first and second cleavage. We did not use time from spawning, fertilization, or egg activation, because the interval between these events and first cleavage includes different developmental events, such as meiotic divisions, in different species.

We used the median times from each treatment to limit the effects of unusually slow embryos. There was little variation among median times within a species at a given temperature. For many species, two or three spawns were recorded at each temperature. The median values for first cell cycle duration and time to first swimming from each treatment were averaged to yield one value per species per temperature for each variable.

For general patterns, we examined the relationship of each variable with time to first swimming in all sampled species with egg diameters less than 250 μm. To remove the potentially large effect of large eggs in one taxon, the four asteroids with very large eggs were included only in analyses of the Asteroidea. For *C. ligatum*, times to rotation are reported in Table 1 and included in Figures 2–3, but are not included in any of the statistical analyses of time to first swimming.

To discern whether any patterns observed were from a few among-clade differences and also to examine evolutionary patterns repeated among clades, we compared species within narrower clades for each continuous variable (egg diameter and cell cycle duration). These included the Spiralia, Echinodermata, Asteroidea, and Urochordata. For the discrete factors (type of locomotion and mode of larval nutrition), we identified several pairwise comparisons that represent independent evolutionary divergences.

Relationships among many phyla are still uncertain (Adoutte *et al.*, 2000). Ideally, one should compare sister taxa that differ in the trait of interest. Sampling metazoan phyla from one geographic region compared species adapted to a similar environment, but limited the number of species that could be sampled from most phyla. Nonetheless, phylogenetic hypotheses from morphological and molecular data (Halanych *et al.*, 1995; Stechmann and Schlegel, 1999; Adoutte *et al.*, 2000) and distributions of traits suggest that our comparisons represent independent evolutionary divergences (Fig. 1). Though recent evidence suggests the lophophorates are polyphyletic (Halanych *et al.*, 1995), the relationships within the group remain uncertain (Adoutte *et al.*, 2000). On the basis of morphology, however, brachiopods, phoronids, and bryozoans have been grouped together as the Lophophorata (Hyman, 1959). Thus, we included comparisons within the lophophorates.

We examined the effect of type of locomotion on time to first swimming in four comparisons of animals with uniciliated or multiciliated cells: the cnidarians *versus* the ctenophore, other lophophorates *versus* the bryozoan, echinoderms *versus* the Lophotrochozoa with multiciliated cells (the bryozoan, nemertean, annelids, and molluscs), and the oweniid *versus* other polychaetes. Occurrence of uniciliated and multiciliated cells is reviewed by Gardiner (1978) and Nielsen (1995). Urochordates with muscular locomotion provide a fifth independent divergence in locomotory structure. For mode of larval nutrition, we compared species with feeding and nonfeeding larvae within the Mollusca, the Echinodermata, and the Lophophorata, and between the Cnidaria and Ctenophora.

Figure 1. A phylogeny of the phyla used in this study. Type of locomotion and mode of larval nutrition are indicated for species sampled. UC = uniciliated cells; MC = multiciliated cells; M = muscles. NF = nonfeeding larva; F = feeding larva. See text for details of independent evolutionary comparisons.

Results

Time to first swimming, egg diameter, and cell cycle duration are summarized in Table 1. The Q_{10} values (a measure of the increase in rate for each 10 °C rise in temperature) for cell cycle duration and time to first swimming ranged from about 2 to 4. Although the effect of temperature varied greatly among species, general trends in the results were consistent at 10 °C and 14 °C. Moreover, there was little differential effect of temperature on different stages of development: the mean of [(time to swimming)/(time for first cell cycle) at 14 °C]/[(time to swimming)/(time for first cell cycle) at 10 °C] for 29 species was 0.999 ± 0.083 SD (n = 29). This sample of species includes all those observed at both 10 °C and 14 °C. The figures depict data at 10 °C. Similar patterns were observed at 14 °C (Table 2).

Although planktonic embryos first swim at an early stage and young age, they have not converged on a similar minimum time to swimming. For the species with egg diameters less than 250 μm, the minimum and maximum times to first swimming were 10.5 and 45.8 h at 10 °C and 7.7 and 28.9 h at 14 °C. Two covariates account for about half of the variation in time to first swimming.

Time to first swimming was significantly correlated with both egg diameter and first cell cycle duration overall,

Table 2

Pearson correlations for data shown in Figure 1

	10 °C		14 °C	
	r	*P*	*r*	*P*
Time to swimming *vs.* Cell cycle duration				
Overall	0.500 (29)	**0.006**	0.568 (28)	**0.002**
Spiralia	0.572 (10)	**0.004**	0.612 (10)	0.060
Echinodermata	0.657 (10)	**0.039**	0.496 (10)	0.145
Asteroidea	0.990 (4) 0.381 (8)	**0.010** 0.352	0.715 (4)	0.271
Urochordata	0.976 (3)	0.138	0.972 (3)	0.152
Time to swimming *vs.* Egg diameter				
Overall	0.502	**0.006**	0.569	**0.002**
Spiralia	0.530	0.115	0.564	0.089
Echinodermata	0.675	**0.032**	0.632	0.050
Asteroidea	−0.772 0.980	0.228 **<0.001**	−0.834	0.166
Urochordata	0.959	0.182	0.940	0.222
Cell cycle duration *vs.* Egg diameter				
Overall	0.196	0.308	0.264	0.175
Spiralia	0.098	0.788	0.093	0.798
Echinodermata	0.081	0.825	−0.180	0.619
Asteroidea	−0.823 0.206	0.177 0.625	−0.607	0.393
Urochordata	0.998	**0.044**	0.994	0.070

Underlined values include the four asteroid species with eggs greater than 250 μm. Sample sizes are indicated in parentheses for the first comparison and are the same in subsequent analyses. Bold type indicates statistical significance ($P < 0.05$).

though these two factors were not consistently correlated with each other (Fig. 2 and Table 2). In a multiple regression of time to first swimming on egg diameter and cell cycle duration, the combined effect of egg diameter and first cell cycle duration explained approximately half of the variation in time to first swimming (at 10 °C: $r^2 = 0.496$, $P < 0.001$, $n = 27$; at 14 °C: $r^2 = 0.598$, $P < 0.001$, $n = 26$). This regression included all species with eggs less than 250 μm except the urochordates. The urochordates were not included in this calculation because of their high correlation between egg diameter and cell cycle duration.

Cell cycle duration

Time to first swimming was positively correlated with cell cycle duration (first to second cleavage) in almost all clades, phyla, and classes considered, but the correlation was not always significant (Table 2). The only exception to the trend was within the polychaetes, because *Owenia fusiformis* has an anomalously long time to first swimming. It may have initially benthic development of clustered eggs (unpubl. obs.).

Egg size

Time to first swimming was positively correlated with egg diameter overall and for the Spiralia, Echinodermata, and Urochordata (Table 2). The positive correlation was not significant in the spiralians or urochordates, perhaps because of the small sample sizes. If the times to rotation for *Calliostoma ligatum* are included, the correlation in the spiralians is significant ($r = 0.659$, $P = 0.028$). Overall, time to first swimming was positively correlated with egg diameter when species with feeding and nonfeeding larvae were considered separately (Table 3). The correlation was negative for asteroids with feeding larvae but positive when species with nonfeeding larvae were included, which greatly increased range in egg sizes and times to swimming (Tables 2 and 3). Within the echinoids there was also a negative correlation for the four species with feeding larvae. Again, this may be due to small sample sizes and a narrow range of egg sizes within these groups.

Egg diameter and cell cycle duration were not correlated with each other overall or for the spiralians or echinoderms (Table 2). Within the urochordates, the correlation coefficient for egg diameter and cell cycle duration was high but not significant with the small sample of three species. For the four species of echinoids, the correlation was negative, but sample size and ranges were small.

Type of locomotion

There was no significant difference in time to swimming among species that swim using uniciliated cells, multiciliated cells, or muscle at either temperature (Kruskal-Wallis test, 10 °C: $H = 0.771$, $P = 0.680$; 14 °C: $H = 0.300$, $P = 0.861$). Similarly, there was no consistent pattern

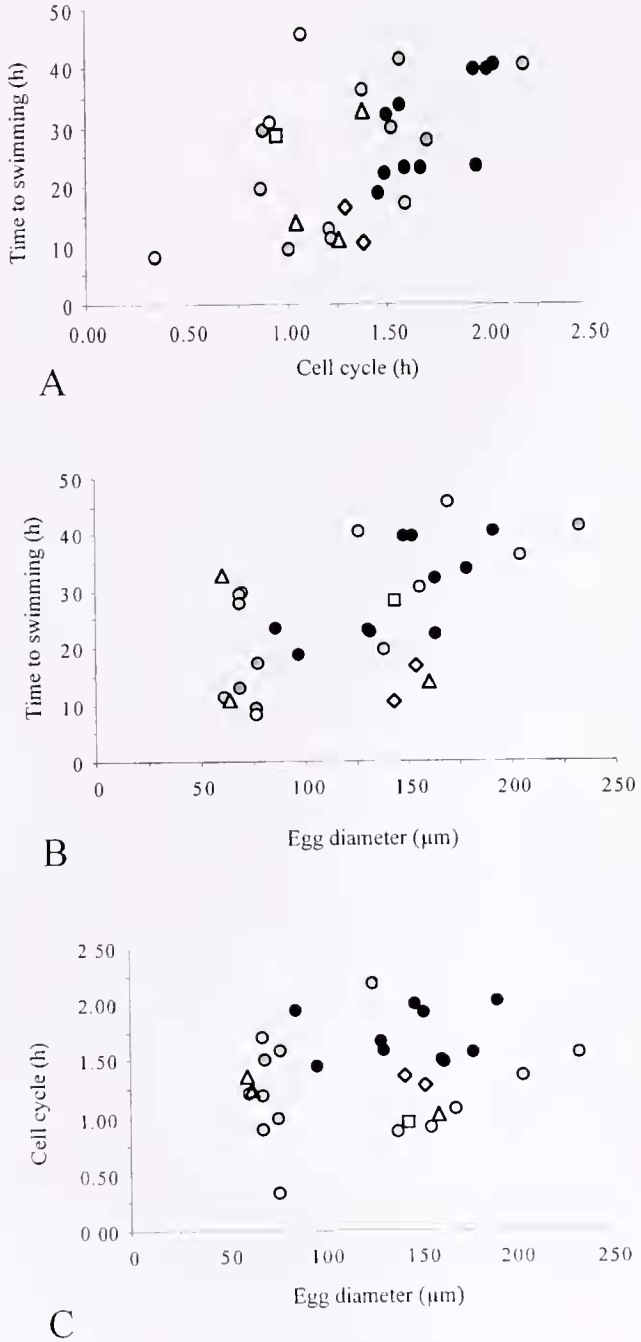

Figure 2. Relationships among time to swimming and two continuous variables at 10 °C. Four asteroid species with eggs greater than 250 μm are not shown. (A) Time to swimming is significantly correlated with cell cycle duration ($P = 0.006$). (B) Time to swimming is significantly correlated with egg diameter ($P = 0.006$). (C) Cell cycle duration is not significantly correlated with egg diameter ($P = 0.308$). Diamonds = Cnidaria; square = Ctenophora; gray circles = Spiralia; triangles = Lophophorata; black circles = Echinodermata; open circles = Urochordata. See Table 2 for statistical analyses.

relating time to first swimming to type of locomotion among pairwise comparisons. The ctenophore with multiciliated cells took 1.7 to 2.7 times longer to develop to swimming than the two cnidarians with uniciliated cells. Within the Lophophorata, the bryozoan with multiciliated cells took 1.7 to 3 times longer to develop to swimming than the phoronid and brachiopod with uniciliated cells. The times to first swimming for echinoderms with uniciliated cells overlapped broadly with those for the Lophotrochozoa with multiciliated cells (echinoderms 18.9 to 40.7 h, lophotrochozoans 9.3 to 40.6 h for 10 °C and eggs less than 250 μm). The three polychaetes with multiciliated cells developed to swimming 1.7 to 3.2 times faster than the polychaete with uniciliated cells. Two of the three urochordates, which swim with muscle, developed to swimming in the same time as species that first swim with cilia. The cell cycles and egg diameters of groups with muscle, multiciliated cells, and uniciliated cells overlapped broadly (Table 1) indicating that the effects of egg diameter and cell cycle were not obscuring a relation between type of locomotion and time to first swimming. Within the pairwise comparisons, the species with the longest time to swimming in each comparison often had an egg diameter and cell cycle duration toward the lower end of the range of species included in the comparison. (An exception occurred in the lophophorates, in which the bryozoan had the longest time to swimming as well as the longest cell cycle.)

Mode of larval nutrition

Times to first swimming for species with feeding larvae and species with nonfeeding larvae were not significantly different at either temperature (Mann-Whitney test, 10 °C: $U = 95$, $P = 0.115$; 14 °C: $U = 89$, $P = 0.783$). For within-clade comparisons, there was no consistent pattern relating time to swimming to type of larval nutrition. The times to swimming for the two molluscs with feeding overlapped those for the three with nonfeeding larvae. The four echinoderms with nonfeeding larvae took longer to develop to swimming than did the nine with feeding larvae. Within the Lophophorata, the times to first swimming for the feed-

Table 3

Pearson correlations for time to first swimming and egg diameter with feeding or nonfeeding larvae

	10 °C		14 °C	
	r	*P*	*r*	*P*
NF overall	0.497 (9)	0.173	0.494 (9)	0.176
F overall	0.614 (20)	**0.004**	0.705 (19)	**0.001**
F Asteroidea	−0.772 (4)	0.228	−0.834 (4)	0.166
NF Asteroidea	0.940 (4)	0.060		

Four asteroid species with eggs greater than 250 μm are not included except in nonfeeding asteroid analyses. Sample size is in parentheses. Bold type indicates statistical significance ($P < 0.05$). NF = nonfeeding larvae. F = feeding larvae.

ing phoronid and bryozoan bracketed that of the nonfeeding brachiopod. The feeding ctenophore took longer to develop to swimming than the two nonfeeding cnidarians.

The cell cycles of groups with feeding and nonfeeding larvae overlapped broadly, indicating that the effect of cell cycle duration was not obscuring a relation between mode of larval nutrition and time to first swimming. The ctenophore took longer to develop to swimming than the cnidarians but had a shorter cell cycle. The bryozoan took longer to swim than the other lophophorates and had a longer cell cycle. The cell cycles of the feeding and nonfeeding molluscs overlapped.

Apparent effects of larval nutrition, where they occur, may be attributable to effects of egg size. In general, egg diameters of species with feeding and nonfeeding larvae overlapped. Within the lophophorate and cnidarian/ctenophore comparisons, the species with the longest time to swimming had an egg diameter at the lower end of the range of egg diameters in the comparison. However, in comparisons of more closely related species, the nonfeeding bivalve and asteroids took longer to develop to swimming than their within-class relatives with feeding larvae, but they also had larger eggs. Overall, time to first swimming was positively correlated with egg diameter in species with feeding and nonfeeding larvae (Table 3). The trend holds in feeding echinoderms and nonfeeding urochordates (Table 2). (The trend was not significant in the urochordates, perhaps because of the small sample size.) However, in feeding echinoids and feeding asteroids, time to first swimming was not positively correlated with egg diameter. In nonfeeding asteroids, the positive correlation was not significant (Tables 2 and 3). This could be a result of the small sample sizes. In any case, it is difficult to separate any effect of mode of larval nutrition from that of egg diameter.

Asteroidea

In analyses of asteroids with eggs less than 250 μm in diameter, time to first swimming was significantly correlated with cell cycle but not with egg diameter (Table 2). However, the range of egg diameters in the comparison (30 μm) may not be large enough to reveal an effect of egg size (Table 1). If the four species with eggs greater than 250 μm are considered as well, time to first swimming was significantly correlated with egg diameter, but not with cell cycle. In this broader comparison of asteroids, the ranges in egg diameters and time to swimming were much larger, but the range in cell cycle durations was not (Table 1). Cell cycle and egg diameter were not significantly correlated with each other in any of the asteroid comparisons. The asteroids with nonfeeding larvae take almost twice as long to develop to swimming as do the asteroids with feeding larvae, and they have much larger egg diameters.

Discussion

Time to first swimming correlated with egg diameter overall and within most groups considered. Time to first swimming also correlated with cell cycle duration (first to second cleavage). Cell cycle duration and egg diameter did not correlate with each other in most of the groups we observed. Thus the hypothesis that egg diameter correlates with time to first swimming simply because larger eggs take longer to divide is rejected.

Species with nonfeeding larvae often have larger eggs than species with feeding larvae (Strathmann, 1987). Though egg size often correlated with time to first swimming in this data set, mode of larval nutrition did not correlate with time to first swimming overall or within most groups considered. Limited indications that species with nonfeeding larvae take longer to develop to swimming were confounded with egg size. The relationship between time to first swimming and mode of larval nutrition remains uncertain, and clarification requires additional independent comparisons of related species with feeding and nonfeeding larvae that overlap in egg size. However, the correlation between time to first swimming and egg diameter appears to be independent of the mode of larval nutrition.

Egg diameter correlates with time to first swimming in ciliated swimmers, presumably because there is a time constraint to making cilia and it takes more cilia to propel a larger object through the water (Emlet, 1994). The ciliated species that we observed are almost spherical when they first swim. The diameter of embryos at first swimming increases with egg diameter. At low Reynolds numbers, the drag on a moving sphere increases with the diameter of the sphere (Vogel, 1994). In a ciliated embryo, one might also expect the number of cilia necessary for swimming to increase as the diameter of the embryo increases (Emlet, 1994). In embryos with uniciliated cells, the number of cilia depends on the number of cells, and the limiting factor may be the construction of new cells. In embryos with multiciliated cells, production of cilia still requires time, even if fewer cell divisions are required. In each case, construction of cilia requires the expression of tubulin genes (Tansey and Ruderman, 1983; Gong and Brandhorst, 1988; Damen *et al.*, 1994). Production of more cilia by larger embryos may delay time to swimming, whether each of many cells makes one cilium or each of few cells makes many cilia.

For species that swim with cilia, large egg diameters may delay swimming while other development proceeds. The four asteroids with very large eggs start swimming nearly 20 h later than the slowest asteroid with eggs less than 250 μm in diameter, and they start to swim as gastrulae rather than as blastulae. Within the Spiralia, species with larger eggs also started swimming at later ages and stages (Table 1).

Cell cycle duration correlated with time to first swimming

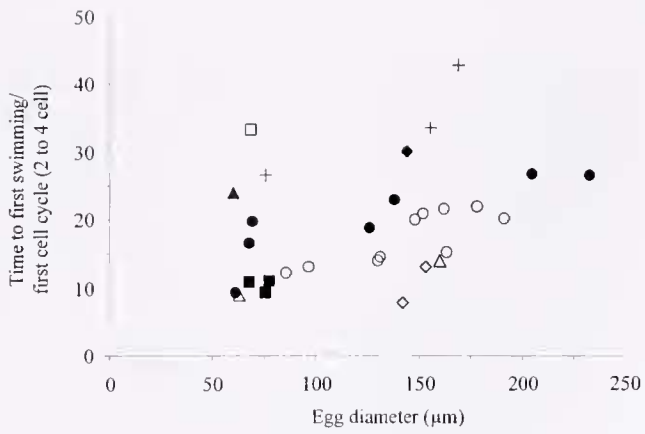

Figure 3. Relationship between the extrapolated maximum number of cell cycles prior to swimming (if all were as brief as the first cell cycle) and egg diameter. Data from 10 °C. Open symbols = uniciliated cells; filled symbols = multiciliated cells; + = muscle. Groups of taxa within these categories are indicated by diamonds = Cnidaria and Ctenophora; squares = Polychaeta; triangles = Lophophorata; + = Urochordata; circles = other species (open = Echinodermata; filled = Mollusca, Nemertea).

overall and within most groups considered. The simplest explanation for this pattern is that the number of cells embryos need to swim determines time to first swimming. The duration of the first cell cycle (first to second cleavage) gives an indication of the maximum speed at which a species' egg can be divided into cells. In general, cell cycles slow as development progresses and transcription increases. If, however, embryos continued to multiply cells at the same rate as in the first cell cycle in all cell lineages, the maximum number of cells at first swimming would be 2^n, where n is the extrapolated number of cell cycles (time to first swimming divided by first cell cycle duration).

These extrapolated maximum cell numbers at swimming range over 10 orders of magnitude in this sample of species. When these extrapolated cell numbers are plotted against egg diameter, the embryos cluster by type of locomotion (Fig. 3). If the cell cycles of *Ascidia paratropa*, an ascidian, continued from fertilization to swimming at the initial rate, the tadpole would have approximately one trillion cells (an average of ~41 cell divisions in a cell lineage) at swimming—an absurdly large number. *Aglantha digitale*, a cnidarian, would have only 200 cells (an average of ~8 divisions in a cell lineage)—a remarkably small number. In general, for a particular egg size, if species sustained their initial rates of cell division through development to swimming, species with uniciliated cells would have fewer cells than those with multiciliated cells, which would have fewer cells than the animals that swim with muscle (Fig. 3). Instead, cell cycles appear to lengthen differentially.

Estimates of cell number indicate that species that swim with different equipment lengthen their cell cycles differently prior to swimming. The number of cells in tadpole larvae of solitary ascidians is fairly constant among species (Yamada and Nishida, 1999). Thus, *Boltenia villosa* and *Ascidia paratropa* probably have approximately 2500–3000 cells at first swimming, implying that, on average, cell lineages undergo only 11 or 12 divisions instead of the projected maximum number of 32 and 42 respectively. Cell cycles lengthen to different extents in other species. Cell counts in two echinoids with uniciliated cells that start swimming as simple blastulae suggest relatively little lengthening of cell cycles. *Dendraster excentricus* has approximately 1200–1500 cells at swimming (K. Tanaka, pers. comm., Univ. of Washington, 2000). *Strongylocentrotus purpuratus* has approximately 350 cells at swimming (Hinegardner, 1967). In each echinoid, the embryos completed about 70% of the maximum number of cell cycles projected. Estimates of cell numbers at first swimming in species with multiciliated cells suggest that they may be intermediate to the echinoderms and urochordates in lengthening later cell cycles (E. Edsinger-Gonzales, pers. comm., Univ. of Utrecht, 2000; Morrill, 1982).

These results point to differences in allocation of time to multiplying compared with differentiating cells that are associated with different means of achieving early swimming. Preliminary comparisons of number of cells at first swimming suggest that animals that first swim as very simple embryos, such as echinoids and cnidarians, may spend a large fraction of their time simply dividing cells, because they have little need for cell differentiation. Species that swim with multiciliated cells often incorporate their cilia into specialized swimming structures. These animals may spend less time simply multiplying cells and more time differentiating them, than do species with uniciliated cells. Similarly, animals that start swimming using muscles (*e.g.*, tunicates, chaetognaths, crustaceans, and fish) may spend even more time differentiating cells relative to multiplying them. Urochordates appear to invest a large proportion of their development time prior to swimming in processes other than multiplying cells. Indeed, developmental studies suggest the neural tube is the only tissue with continuing cell division after gastrulation. Muscle and notochord lineages stop dividing after the 9th cell cycle and differentiate (Nishida, 1997). Cell counts for other animals at first swimming could test the hypothesis that mode of locomotion affects a trade-off between differentiation and multiplication of cells.

In our sample of animals with planktonic development, the urochordates had the shortest cell cycles at a given time to first swimming. The short cell cycles and other developmental devices permit swimming as a tadpole (with muscles, notochord, and nerves) as early as other animals swim as a hollow ball of ciliated cells. Selection for early swimming with muscles may have selected for especially short early cell cycles in the urochordates. Comparisons with other planktonic embryos that develop muscles for first

swimming, as in copepods and chaetognaths, could test the hypothesis that short early cell cycles have generally evolved as part of rapid development to swimming with muscles.

The high correlation between egg diameter and cell cycle duration in the urochordates, though nonsignificant in this sample of three species, is consistent with past studies (Berrill, 1935). If egg size and cell cycle duration are correlated only for embryos with the shortest cell cycles, then possibly egg size does set the lowest limits on early cell cycle durations. The rate of these concentrated cell divisions may be limited by cellular factors related to egg size, for example, construction of the cytoskeleton; and evolution may have shortened the early cell cycles to a limit imposed by egg size. However, asteroids with eggs that are larger by a factor of 6 have cell cycles that are only twice as long as those of urochordates (Table 1).

The combined effect of egg diameter and early embryonic cell cycle explained nearly half of the variation in time to swimming in this diverse group of animal embryos. Many factors could account for the remaining variation. Differences in vulnerability among embryos may explain some of the variation. Protected embryos begin swimming at a later age and stage than their less protected relatives (Strathmann *et al.*, 2002). Though among the least protected of marine invertebrate embryos, planktonic embryos differ in buoyancy, extra-embryonic membranes, and chemical defenses (Szollosi, 1969; Lucas *et al.*, 1979; Strathmann, 1987; Young, 1995; Lindquist, 1996; McClintock and Baker, 1997).

Within several groups, related animals start to swim at very different ages and stages. In planktonically developing gastropods, for example, both *Tectura scutum* and *Calliostoma ligatum* have formed trochophores when their cilia start to beat. However, *T. scutum* swims as a trochophore nearly a day before *C. ligatum* starts to rotate in its capsule, and it rotates in its capsule for several days before hatching as a veliger. The planktonically developing abalones, such as *Haliotis kamtschatkana*, also hatch as trochophores (Strathmann, 1987). *Tegula funebralis* (like *C. ligatum* a trochoidean from the northeast Pacific) has a 1-h cell cycle from first to second cleavage at 13–15 °C (like *C. ligatum*) but hatches as a pretorsional veliger at 40 h, a much earlier stage and age than for *C. ligatum* (Moran, 1997). Perhaps the egg capsule of *C. ligatum* provides protection that relieves selection for early swimming. Rotation of *C. ligatum* in its capsule for several days before hatching suggests that early ciliary motion may have benefits other than swimming. Ancestral benthic development may account for the long time to first swimming for the free-spawning *C. ligatum*. Some species of trochoidean gastropods have benthic egg masses, and others have individual planktonic embryos (Hadfield and Strathmann, 1990). Evolutionary transitions between benthic egg masses and single planktonic embryos could have occurred many times in this group. More extensive comparisons could test the hypothesis that a late stage at hatching of a planktonic embryo is associated with a more protected development in the ancestry.

Times to first swimming for diverse embryos of distantly related species were remarkably similar. The comparisons presented here represent extremely ancient divergences among clades of animals. The structures formed for first swimming are highly disparate. Nonetheless, major structural and functional differences in embryos and larvae appear to have little influence on embryonic durations. Instead, egg size and cell cycle length appear to most reliably predict times to swimming for these animals. However, as discussed above, the trade-offs and devices that allowed such convergence in times to swimming have shaped the development and life histories of major groups of metazoans. The evolution of early swimming is associated with the evolution of cell cycle durations, timing of transcription and differentiation in cell lineages, parental investment in protection of offspring, developmental stage at first locomotion, and first locomotory capabilities.

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